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# Substituted 5-Nitro-1,3-dioxanes: Correlation of Chemical Structure and Antimicrobial Activity


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**Abstract** □ Various derivatives of 5-nitro-1,3-dioxane were synthesized to determine the relative effect of chemical substitution in the 2- and 5-positions on broad spectrum antimicrobial activity. Each compound was evaluated quantitatively by calculation of a microbiocidal index, which measured the time to kill several different microorganisms. This test system indicated that 5-bromo-5-nitro substitution was essential for significant activity. Optimal activity was effected by 2-methyl substitution in the alkyl series and 2-hydroxyphenyl substitution in the aryl series. The antimicrobial activity of the substituted dioxanes was not related directly to water solubility or hydrolysis to microbiocidal diols or aldehydes.



**Keyphrases** □ Dioxanes, substituted—series synthesized, effect of substituents on antimicrobial activity □ Antimicrobial activity—series of substituted dioxanes synthesized, effect of substituents evaluated □ Structure–activity relationships—substituted dioxanes synthesized, effect of substituents on antimicrobial activity


Chemical agents that prevent microbial growth are essential ingredients for various topical products (1–3). The recent increased awareness of the potential health hazard of microbiologically contaminated pharmaceutical and cosmetic preparations (4–6) initiated a trend toward greater regulatory requirements for these products (7, 8). This turn of events emphasized the urgent need for more effective preservatives.

Several reviews described the significant factors contributing to the optimal efficacy of antimicrobial agents (9–11). Ideally, a preservative for topical preparations should be effective over a wide pH range (pH 5–9) and be physically and chemically compatible with the formulation ingredients. Furthermore, this agent should not be irritating, sensitizing, or absorbed to toxic levels after application.

To satisfy these requirements, chemical modification of the antimicrobial agent is often necessary. The design of the microbial test system to reflect significant differences in the activity of these modified compounds becomes a critical factor in their evaluation (12–14).

Various nitro and halonitro aliphatic alcohols exhibit antimicrobial activity and synergism (15–23). In particular, 2-bromo-2-nitro-1,3-propanediol is highly effective at low concentrations against many bacteria, yeasts, and molds. However, its inhibitory activity decreases as the pH increases from 5.3 to 7–8 (24). Its utility as a preservative was demonstrated in cosmetic formulations (25).

Various substituted 1,3-dioxanes also had antifungal and antibacterial properties. In some instances, this activity was attributed to ring cleavage and subsequent release of the antimicrobial aldehyde or alkane diol (26–28). However, many interesting dioxanes, including nitro and halonitro derivatives, are stable in the 5–9 pH range (29–35). Most notable is 5-bromo-5-nitro-1,3-dioxane, which exhibits broad spectrum antimicrobial activity and is an effective preservative in topical formulations (36).

In this study, derivatives of 5-nitro-1,3-dioxane were synthesized to determine the relative effect of various substituents in the 2- and 5-positions on broad spectrum antimicrobial activity.

## EXPERIMENTAL

The chemical structures, physical constants, methods of preparation, and microbiocidal index for the compounds used are listed and referenced in Table I.

**Chemical Synthesis**—*Method A: 5-Bromo-5-nitro-1,3-dioxane (I)*—In accordance with the procedure of Wessendorf and Bellinger (36), a mixture of 50.5 g (0.25 mole) of 2-bromo-2-nitropropane-1,3-diol, 7.5 g of paraformaldehyde (equivalent to 0.25 mole of formaldehyde), and 12.5 g of polyphosphoric acid was heated at 100° for 3 hr. Then the mixture was cooled and extracted with methylene chloride. This extract was washed with 5% sodium bicarbonate solution, dried over anhydrous sodium sulfate, and evaporated to dryness, yielding 36 g of I, mp 49–50°.

*Method B: 5-Bromo-2-methyl-5-nitro-1,3-dioxane (II)*—Forty grams (0.2 mole) of 2-bromo-2-nitropropane-1,3-diol was slurried with 0.3 g of *p*-toluenesulfonic acid and 200 ml of benzene. Acetaldehyde (13.5 g, 0.3 mole) was added gradually and stirred for 30 min at room temperature. Then the mixture was refluxed until 4 ml of water was collected in a Dean–Stark trap during 1.5 hr.

The reaction mixture was cooled and subsequently washed with 5% sodium bicarbonate solution. The washed residue was dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo*. The residual oil was distilled at 72°/0.2 mm to yield 37 g (85%) of colorless liquid (II).

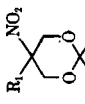
*Method C: 5-Bromo-2,2-dimethyl-5-nitro-1,3-dioxane (VII)*—A solution of 100 g (0.5 mole) of 2-bromo-2-nitropropane-1,3-diol was prepared in 110 ml (1.5 moles) of acetone. To this solution, 65 ml (0.5 mole) of boron fluoride etherate was added rapidly with stirring. After being stirred for 10 min, the clear solution was poured into a mixture of 550 ml of a saturated solution of sodium bicarbonate and ice. The resultant crude product was isolated by filtration, dried, and recrystallized with water from ethanol solution, yielding 62 g (51%) of VII, mp 79–81°.

*Method D: 5-Bromo-2-(m-hydroxyphenyl)-5-nitro-1,3-dioxane (XII)*—A suspension containing 50 g (0.25 mole) of 2-bromo-2-nitropropane-1,3-diol and 0.4 g of *p*-toluenesulfonic acid in 200 ml of dry benzene was stirred and refluxed with heat through a Dean–Stark trap. A solution of 24.4 g (0.20 mole) of *m*-hydroxybenzaldehyde in 50 ml of dioxane was added dropwise to the refluxing mixture during 1 hr. After collection of 3.4 ml of water, the reaction mixture was cooled and the benzene was evaporated at reduced pressure.

The residue was dissolved in 200 ml of ethyl acetate and washed with two 100-ml portions of 10% sodium bisulfite solution. The ethyl acetate layer was separated, dried over anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. The crude product was crystallized from hot benzene to yield 35 g (57%) of crystalline XII, mp 118–119°.

*Method E: 5-Bromo-2-methoxy-5-nitro-1,3-dioxane (XV)*—A suspension of 40 g (0.2 mole) of 2-bromo-2-nitropropane-1,3-diol in

Table I—Chemical and Microbiocidal Properties



| Compound | $R_1$ | $R_2$ | $R_3$ | Melting Point (Boiling Point) | Empirical Formula | Analysis, % | Calc. | Found | Method[a] of Preparation | Microbiocidal[b] Index |
|---|---|---|---|---|---|---|---|---|---|---|
| I | Br | H | H | 49–50°C | — | — | — | — | A | 1275 |
| II | Br | H | $CH_3$ | (72°/0.25 mm) | $C_5H_8BrNO_4$ | C | 26.57 | 26.47 | B | 1375 |
| | | | | | | H | 3.57 | 3.74 | | |
| | | | | | | Br | 35.35 | 35.31 | | |
| | | | | | | N | 6.20 | 6.13 | | |
| III | Br | H | $C_2H_5$ | 58–59° | $C_6H_{10}BrNO_4$ | C | 30.02 | 30.11 | B | 1350 |
| | | | | | | H | 4.20 | 4.08 | | |
| | | | | | | Br | 33.29 | 33.39 | | |
| | | | | | | N | 5.83 | 5.60 | | |
| IV | Br | H | $C_3H_7$ | (73–75°/0.005 mm) | $C_7H_{12}BrNO_4$ | C | 33.10 | 33.32 | B | 1275 |
| | | | | | | H | 4.76 | 4.63 | | |
| | | | | | | Br | 31.45 | 31.64 | | |
| | | | | | | N | 5.50 | 5.66 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| V | Br | H | $CH(CH_3)_2$ | 42–44° | $C_7H_{12}BrNO_4$ | C | 33.10 | 32.87 | B | 1300 |
| | | | | | | H | 4.76 | 4.63 | | |
| | | | | | | Br | 31.45 | 31.66 | | |
| | | | | | | N | 5.50 | 5.24 | | |
| VI | Br | H | $C_{10}H_{21}$ | 55–58° | $C_{14}H_{26}BrNO_4$ | C | 47.73 | 47.98 | B | 75 |
| | | | | | | H | 7.44 | 7.67 | | |
| | | | | | | Br | 22.68 | 22.48 | | |
| | | | | | | N | 3.98 | 4.15 | | |
| VII | Br | $CH_3$ | $CH_3$ | 79–81°[d] | — | | — | — | C | 1175 |
| VIII | Br | $R_2R_3$ | $(CH_2)_5$ | 86–89° | $C_9H_{14}BrNO_4$ | C | 38.55 | 38.61 | C | 0 |
| | | | | | | H | 5.00 | 4.80 | | |
| | | | | | | Br | 28.60 | 28.59 | | |
| | | | | | | N | 5.00 | 4.92 | | |
| IX | Br | H | $C_6H_5$ | 82–84°[e] | — | | — | — | B | 825 |
| X | Br | $CH_3$ | $C_6H_5$ | 87° | $C_{11}H_{12}BrNO_4$ | C | 43.73 | 43.48 | C | 950 |
| | | | | | | H | 4.00 | 3.92 | | |
| | | | | | | Br | 26.45 | 26.83 | | |
| | | | | | | N | 4.64 | 4.66 | | |
| XI | Br | H | $p$-$ClC_6H_4$ | 103°[f] | — | | — | — | B | 0 |
| XII | Br | H | $m$-$HOC_6H_4$ | 118–119° | $C_{10}H_{10}BrNO_5$ | C | 39.50 | 39.72 | D | 1300 |
| | | | | | | H | 3.31 | 3.43 | | |
| | | | | | | Br | 26.28 | 26.06 | | |
| | | | | | | N | 4.61 | 4.55 | | |
| XIII | Br | H | $p$-$HOC_6H_4$ | 142–144°[g] | — | | — | — | D | 1375 |
| XIV | Br | H | $CH{=}CHC_6H_5$ | 105–112° | $C_{12}H_{12}BrNO_4$ | C | 45.88 | 45.72 | B | 0 |
| | | | | | | H | 3.85 | 3.86 | | |
| | | | | | | Br | 25.44 | 25.48 | | |
| | | | | | | N | 4.46 | 4.56 | | |
| XV | Br | H | $OCH_3$ | 88–90° | $C_5H_8BrNO_5$ | C | 24.81 | 24.64 | E | 1150[l] |
| | | | | | | H | 3.33 | 3.22 | | |
| | | | | | | Br | 33.01 | 33.26 | | |
| | | | | | | N | 5.79 | 5.59 | | |
| XVI | Br | $R_2R_3$ | =O | 142° | $C_4H_4BrNO_5$ | C | 21.26 | 21.47 | F | 1325[l] |
| | | | | | | H | 1.78 | 1.84 | | |
| | | | | | | Br | 35.36 | 35.20 | | |
| | | | | | | N | 6.20 | 6.41 | | |
| XVII | Cl | H | $CH_3$ | Liquid | $C_5H_8ClNO_4$ | C | 33.07 | 33.31 | G | 150 |
| | | | | | | H | 4.44 | 4.50 | | |
| | | | | | | Cl | 19.53 | 19.26 | | |
| | | | | | | N | 7.71 | 7.90 | | |
| XVIII | Cl | $CH_3$ | $CH_3$ | 72–73°[h] | — | | — | — | G | 0 |
| XIX | $CH_3$ | H | H | 67–69°[i] | — | | — | — | C | 0 |
| XX | H | H | $CH_3$ | 73–74° | $C_5H_9NO_4$ | C | 41.10 | 40.92 | H | 0 |
| | | | | | | H | 5.52 | 5.47 | | |
| | | | | | | N | 9.59 | 9.42 | | |
| XXI | $CH_2OH$ | H | $CH_3$ | (92–94°/0.025 mm) | $C_6H_{11}NO_5$ | C | 40.68 | 40.56 | B | 0 |
| | | | | | | H | 6.26 | 5.96 | | |
| | | | | | | N | 7.91 | 7.92 | | |
| XXII | $CH_2OH$ | $CH_3$ | $CH_2Cl$ | 114–116°[j] | — | | — | — | C | 0 |
| XXIII | $CH_2N(CH_3)_2$ | $CH_3$ | $CH_3$ | 81–83°[k] | — | | — | — | I | 225 |
| XXIV | 2-Bromo-2-nitropropane-1,3-diol | | | — | — | | — | — | | 1200[m] |

[a]Methods under *Experimental*. Elemental and NMR analyses for all compounds were consistent for their chemical structures. [b]Maximum score is 1400. [c]Lit. (36) mp 49°. [d]Lit. (39) mp 81°. [e]Lit. (34) mp 87°. [f]Lit. (34) mp 105–106°. [g]Lit. (34) mp 137–139°. [h]Lit. (39) mp 73–74°. [i]Lit. (40) mp 71°. [j]Lit. (32) mp 114.5°. [k]Lit. (37) mp 87°. [l]Significant activity attributed to hydrolysis to XXIV. [m]Obtained commercially from Goldschmidt Chemical Co.

Table II—Water Solubility *versus* Microbiocidal Activity

| Compound | Solubility (25°), % | Microbiocidal Index |
|---|---|---|
| II | 0.613 | 1375 |
| I | 0.577 | 1275 |
| VII | 0.105 | 1175 |
| III | 0.077 | 1350 |
| XIII | 0.043 | 1375 |
| IX | 0.046 | 825 |
| IV | 0.028 | 1275 |

250 ml of benzene was treated with 0.3 g of *p*-toluenesulfonic acid and 21.2 g (0.2 mole) of trimethyl formate. The agitated mixture was refluxed through a Dean–Stark trap until 120 ml of distillate was collected. The reaction mixture was evaporated to dryness, and the solid mass was recrystallized from absolute methanol, yielding 20 g (41%) of XV as dense acicular crystals, mp 88–90°.

*Method F: 5-Bromo-5-nitro-1,3-dioxan-2-one (XVI)*—A solution of 40 g (0.2 mole) of 2-bromo-2-nitropropane-1,3-diol was prepared in 100 ml of pyridine and cooled to 0°. Phosgene was bubbled slowly into the vigorously stirred reaction mixture for 45 min. The resultant viscous mixture was diluted with 600 ml of ether, and a tacky solid mass was produced. This residue was removed by decanting the liquid phase and then slurried in 200 ml of water. The crude product was filtered, air dried, and recrystallized twice from hot ethyl acetate, producing 10 g (22% yield) of XVI, mp 132°.

*Method G: 5-Chloro-2-methyl-5-nitro-1,3-dioxane (XVII)*—A solution containing 13.5 g (0.25 mole) of sodium methylate in 75 ml of absolute methanol was added slowly at room temperature to a stirred solution containing 35.4 g (0.20 mole) of 5-hydroxymethyl-2-methyl-5-nitro-1,3-dioxane in 75 ml of absolute methanol. After standing overnight, the mixture was diluted with 400 ml of ether. The sodium salt of 2-methyl-5-nitro-1,3-dioxane was precipitated, isolated by filtration, and dissolved in 150 ml of water. This solution was cooled to 0° in an ice bath and then saturated with chlorine gas.

The resultant oil layer was isolated using a separator and dissolved in 50 ml of chloroform. This solution was washed with water and subsequently dried over anhydrous sodium sulfate. The residue from the evaporation of chloroform yielded 10 g (55%) of pure XVII as a pale-green liquid.

*Method H: 2-Methyl-5-nitro-1,3-dioxane (XX)*—A solution of 20 g (0.12 mole) of L-cysteine hydrochloride in 200 ml of water was buffered to pH 6.0 with sodium bicarbonate. To this solution was added 11.3 g (0.05 mole) of 5-bromo-2-methyl-5-nitro-1,3-dioxane with vigorous stirring. L-Cystine precipitated, and the pH decreased rapidly to 2.3. Sodium bicarbonate was added to increase the pH to 7.5, and stirring was continued for 12 hr.

L-Cystine was removed by filtration and washed with 200 ml of chloroform; the wash solution was used to extract the filtrate. After drying over anhydrous sodium sulfate, the chloroform extract was evaporated to dryness. Recrystallization of the residue from benzene–heptane produced 5.5 g (75% yield) of XX as colorless needles, mp 73–74°.

*Method I: 2,2-Dimethyl-5-dimethylaminomethyl-5-nitro-1,3-dioxane (XXIII)*—In accordance with the procedure of Malinowski and Urbanski (37), 10.0 g (0.05 mole) of 2,2-dimethyl-5-hydroxymethyl-5-nitro-1,3-dioxane was stored in a closed container for 9 days with 35 ml of 40% aqueous dimethylamine. The resultant crystalline product (XXIII) was isolated by filtration and recrystallized from ethanol as dense prisms, yielding 5.0 g (45%), mp 81–83°.

**Microbiological Methods**—A preliminary challenge test was used to determine the antimicrobial potential of the test compounds. Since concentration gradients of antimicrobial suspensions can exhibit a dose response (38), each compound was prepared for testing as a solution or suspension at 0.1 and 0.5% (w/v) in sterile distilled water. Ten milliliters of each concentration was inoculated separately in test tubes with 0.1 ml of a suspension of *Pseudomonas aeruginosa* (ATCC 9027) and *Asperigillus niger* (ATCC 16404) containing $10^8$ organisms/ml.

Plate counts were made initially and at 1, 7, 14, 21, and 28 days using a modified USP procedure (8) for efficacy testing. The incubation temperature of the test solution was reduced from 30 to 25°, and Sabouraud dextrose agar was substituted for soybean–casein digest agar for plating of *A. niger*. Compounds that reduced the counts for both organisms to less than 100 within 14 days at 0.1 and 0.5% concentrations were retested similarly at 0.05 and 0.1%, using the following microorganisms: *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 16231), *Aspergillus niger* (ATCC 16404), *Aspergillus flavus*[1], and *Streptococcus faecalis* (ATCC 8043).

The previous test regimen was followed. The Sabouraud dextrose agar was used only for *A. niger, A. flavus,* and *C. albicans;* all other organisms were cultured in the soybean–casein agar.

## RESULTS AND DISCUSSION

Some substituted dioxanes were prepared from known antimicrobial alcohols and aldehydes. To evaluate the antimicrobial activity of the intact dioxane, all compounds were studied by conventional analytical methods for potential hydrolysis of the dioxane ring in water at 25°. Compound XV was hydrolyzed immediately to the antimicrobial diol (XXIV). Similarly, XVI was hydrolyzed to XXIV but at a slower rate. The percentage decomposition ($t_{\%}$) with time at 25° was $t_{20}$ = 2 hr, $t_{50}$ = 8 hr, and $t_{90}$ = 32 hr. All other compounds revealed no significant hydrolysis of the dioxane ring.

The antimicrobial effectiveness of each compound was quantitated by calculation of the microbiocidal index from the challenge test data using seven organisms at 0.1 and 0.05% (w/v) concentrations. A value of 100, 75, 50, or 25 was assigned to any compound that reduced each organism to a count of less than 100/ml within 1, 7, 14, or 28 days, respectively. The maximum score possible was 1400. Compounds that were ineffective in the initial challenge test at 0.1 and 0.5% (w/v) against *P. aeruginosa* and *A. niger* were scored zero in the microbiocidal index.

The data in Table I indicate that II and XIII were the most active antimicrobials, with scores of 1375. Compounds I, III–V, XII, XIII, and XVI also were highly effective, since all organisms were reduced to less than 100/ml within 7 days. Compounds VII and XV were effective against all organisms within 14 days.

The presence of bromine in the 5-position appears to be essential to significant antimicrobial activity, as noted with I–V, VII, IX, X, XII, and XIII. Replacement of bromine with chlorine (XVII and XVIII) produced a marked reduction of activity; however, hydrogen (XX) or methyl (XIX) in the 5-position resulted in a greater loss of activity. Similar substitutions with the hydroxymethyl group (XXI) did not induce efficacy, and a tertiary amine (XXIII) had only slight antimicrobial action.

In conjunction with 5-bromo substitution, the highest activity for alkyl additions in the 2-position was noted with monosubstituted, minimal chain length (II); the activity decreased with an increase in molecular weight (III–VI). Dialkyl substitution with minimal chain length (dimethyl, VII) was slightly more effective than the 2-phenyl (IX) or 2-phenyl-2-methyl (X) derivatives. However, 2-hydroxyphenyl derivatives (XII and XIII) possessed activity equivalent to the most active alkyl-substituted compound (II). The introduction of 2-*p*-chlorophenyl (XI) and 2-styryl (XIV), which were substituents in certain antifungal dioxanes (34), resulted in marked reductions of antimicrobial properties.

Hydrolysis of XV (2-methoxy) and XVI (2-one) yielded the antimicrobial agent 2-bromo-2-nitropropane-1,3-diol (XXIV). As noted in Table I, the microbiocidal index of this diol was 1200; the values for XV and XVI were 1150 and 1325, respectively. No evidence of synergism was observed for these compounds and their hydrolysates when tested for zone inhibition against *Bacillus subtilis* (ATCC 6633). These data indicate that XVI contributes significantly to the microbiocidal action prior to its hydrolysis. Any contribution by XV to activity was not evident, since it hydrolyzed considerably faster than XVI.

GC analysis was used to determine the water solubility at 25° for some active compounds to determine the relationship of this parameter to microbiocidal activity. These data are given in decreasing order of solubility in Table II. Although it is generally accepted that some degree of water solubility is essential to activity, this comparison with substituted 5-bromo-5-nitro-1,3-dioxanes indicates that no direct relationship exists between the percent water solubility and the degree of antimicrobial activity.

This structure–activity relationship reveals a wide range in antimicrobial action due to chemical modification of the 5-nitrodioxane moiety. The introduction of bromine in the 5-position produced a high

[1] Laboratory culture.

degree of activity. The significance of further substitution in this comparative study is realized since some 2-monoalkyl and 2-hydroxyphenyl derivatives of the active 5-bromo-5-nitro-1,3-dioxane showed increased efficacy. These compounds offer some degree of selectivity to satisfy the multitude of antimicrobial requirements set forth for cosmetic and topical pharmaceutical products.

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# GLC and NMR Analysis of Isomeric Impurities in the New Anti-Inflammatory Agent Benoxaprofen


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**Abstract** □ GLC and NMR methods are described for the determination of four possible isomeric impurities in the novel anti-inflammatory agent benoxaprofen. The 2- and 3-chlorophenyl isomers were determined by GLC after alkaline hydrolysis and subsequent methylation. A rapid NMR procedure, using the lanthanide shift reagent tris- (1,1,1,2,2,3,3-heptafluoro- 7,7-dimethyl- 4,6-octanedionato)-europium, was developed for the 6- and 7-(α-methylacetic acid) isomers. Similar methodology, with tris-(3-heptafluorobutyryl-*d*-camphorato)europium, enabled the determination of the enantiomer ratio for benoxaprofen. For the positional isomers, the limits of detection were 0.05% by GLC and 0.2% by NMR.



**Keyphrases** □ Benoxaprofen—with four isomeric impurities, GLC and NMR analyses □ GLC—analysis, benoxaprofen and isomeric impurities □ NMR—analysis, benoxaprofen and isomeric impurities □ Anti-inflammatory agents—benoxaprofen and four isomeric impurities, GLC and NMR analyses


Recently, the syntheses and anti-inflammatory activity of a number of 2-aryl-5-benzoxazoleacetic acids were described (1). The most active member of the series, 2-(4-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid (benoxaprofen, I), is several times more potent than phenylbutazone in the rat paw edema test and is currently under clinical evaluation.

The material for toxicology and clinical requirements was prepared in a 10-step synthesis (Scheme I).

Isomeric impurities, which can arise at Steps 3 and